Supporting Information

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SI Materials and Methods

Cells, Viruses, Antibodies, and Reagents. HEK293, HeLa, HT1080, 2FTGH-ISRE, and Vero cells were cultured in DMEM supplemented with 10% (vol/vol) FBS, 5 μg/mL penicillin, and 10 μg/mL streptomycin. 293 cells stably expressing HA-tagged ERIS or FLAG-tagged hTLR3 were selected with G418 (0.5 mg/mL). VSV (Indiana strain) was propagated and amplified by infection of a monolayer of Vero cells. Twenty-four hours after infection, the supernatant was harvested and clarified by centrifugation. Viral titer was determined by plaque assay on Vero cells. Sendai virus (C.Z., Wuhan University, Wuhan, China) and NDV-GFP virus (C. Wang, Shanghai Institutes for Biological Sciences, Shanghai, CAS) were obtained as indicated. Poly(I:C) was purchased from Amersham. Poly(dA-dT)·Poly(dT-dA) was synthesized as 5′-d$(AT)_{30}$-3′ and annealed by heating to 95 °C and cool down to room temperature. Lipid transfection was done with GenEscort (Wisegen). To generate mouse polyclonal antibody against human ERIS, cDNA containing residues 219–379aa of human ERIS was cloned into pET30c (Novagen) and protein was purified from *E. coli* by nickel column chromatography. The recombinant protein was injected into mice to produce antisera. Anti-Flag (Sigma), anti-HA (Sigma), anti-VSV (Sigma), anti-ubiquitin (Santa Cruz), anti-GAPDH (Santa Cruz), anti-IKKα (Santa Cruz), anti-calnexin (Bioworld), anti-EEA1 (Cell Signaling), and anti-caspase3 (Transduction Laboratories) antibodies were purchased as indicated.

Plasmids. Flag-tagged and HA-tagged ERIS was constructed by cloning ERIS into p3 × FLAG-CMV-14 (Sigma) or pcDNA3.1 vector (Invitrogen), respectively. Red fluorescent protein (RFP)-tagged ERIS was constructed by cloning ERIS into pTagRFP-N (Evrogen). Deleted, truncated, and point mutants were generated by the QuikChange site-directed mutagenesis kits with pfu-ultra as the polymerase (Stratagene) and the construct coding for the WT protein as the template. Each mutation was confirmed by sequencing. GyrB was amplified from *E. coli* genomic DNA with the following primers: 5′-AATTCTTATGACTCCTCCAGTATC-3′ and 5′-TTACAGGGTACGGGTCATCGCC-3′. Plasmid coding rat Myc-CD8α was kindly provided by Lan Bao (Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (CAS)). Myc-CD8α-RIR/AIA was made through the ligation of annealed primers 5′-ggccgcagagctccaggcccggattcgaacttacaatt-3′ and 5′-ctagaattgtaagttcgaatccgggcctggagctctgc-3′ for Myc-CD8α-RIR and 5′-ggccgcagagctccaggccgcgattgcaacttacaatt-3′ and 5′-ctagaattgtaagttgcaatcgcggcctggagctctgc-3′ for Myc-CD8α-AIA. pEGFP-KDEL was kindly provided by Hiroko Bannai (University of Tokyo, Tokyo, Japan). For reporter assay, pISRE-Luc (Stratagene) and pNifty-Luc (Invivogen), NF-κB–dependent E-selectin–luciferase reporter plasmids, were purchased as indicated. P561-Luc was kindly provided by Ganes C. Sen (Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH). Other promoters were cloned into pGL3-Basic vector (Promega) with the following promoter regions: −1–155 for mIFN-β, −1–649 for mIFN-α1, −1–332 for mIFN-α2, −1–476 for mIFN-α4, −1–1,213 for hIFN-α8, −3–1,592 for mIL-10, −314–58 for mIL-12, −1–1,765 for mIL-22, −1–1,280 for mIL-23, −1–489 for mIL-28, and −1–1,588 for m-inducible NOS. All the constructs were verified by sequencing.

RT-PCR. IFN-β (*Ifnb1*), ISG15 (*Glp2*), IFIT1(*Ifig1*), IP10 (*Cxcl10*), regulated on activation normal T-cell exposed and secreted (RANTES; *ccl5*), and hypoxanthineguanine phosphoribosyl transferse (HPRT) (*Hprt1*) cDNAs were amplified with the following primers: 5′-CCAACAAGTGTCTCCTCCAA-3′ and 5′-ATAGTCTCATTCCAGCCAGT-3′ for IFN-β, 5′-CTTTGCCAGTACAGGAGCTTG-3′ and 5′-GCAGATTCATGAACACGGTGC-3′ for ISG15, 5′-CCACAGCTTACACCATTGGCTG-3′ and 5′-GGGTCCACTTCAAGCACCTTTTC-3′ for IFIT1, 5′-AACCACAAGCACCAAAGCAGAG-3′ and 5′-GTTGATGTTGAAGAGGGCACCT-3′ for IP10, 5′-CAGAGGATTCCTGCAGAGGATC-3′ and 5′-GACTGCTGGGTTGGAGCACTT-3′ for RANTES, and 5′-GGACAGGACTGAAAGACTTGCTCG-3′ and 5′-TCCAACAAAGTCTGGCCTGTATCC-3′ for HPRT.

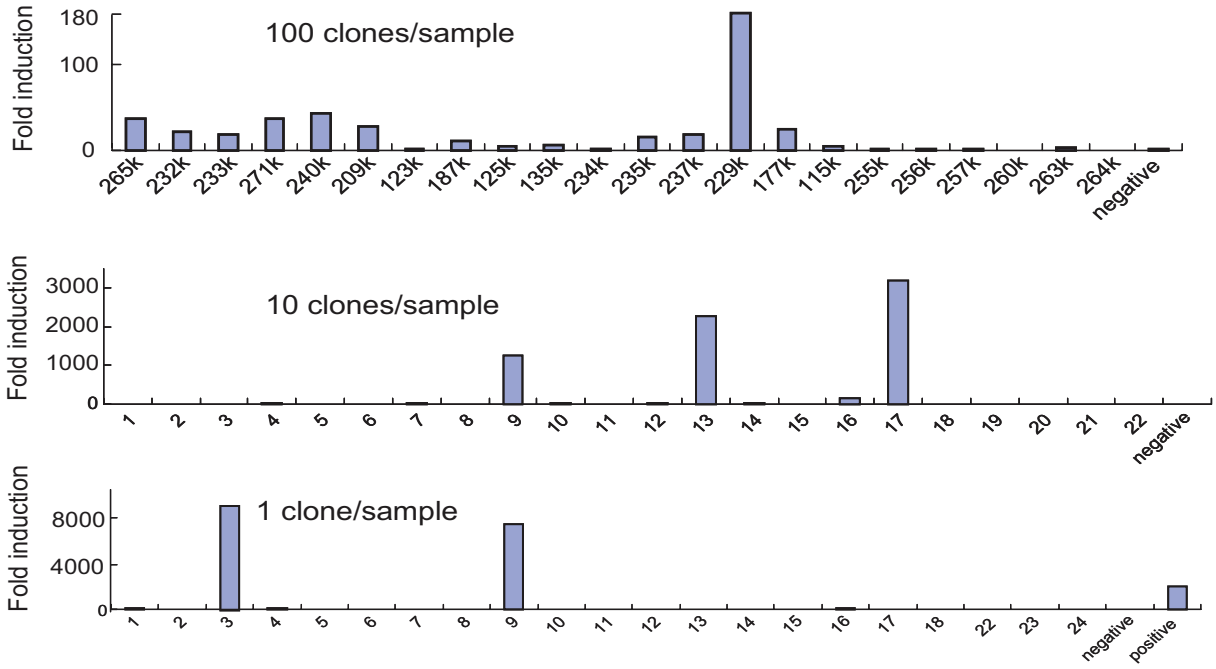

Fig. S1. Identification of ERIS as a strong stimulator of IFN-β. The positive cDNA pool containing $\approx$100 cDNAs, no. 229k, was subdivided into 10 clones per sample and then 1 clone per sample to isolate the single clone responsible for IFN-β induction.

Identities: 68%, Similarity: 81% between *Mus musculus* and *Homo Sapiens*

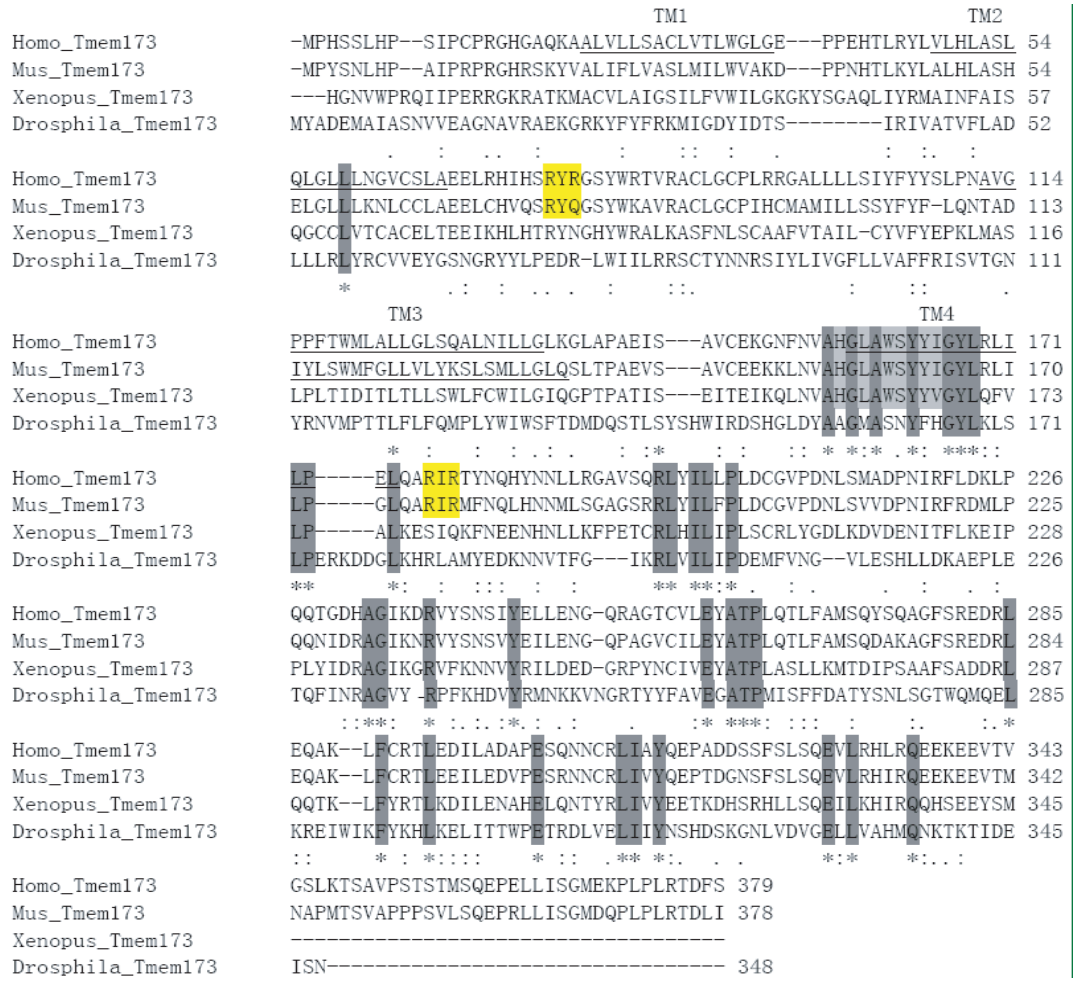

Fig. S2. Alignment of the coding sequences of ERIS. Sequences from human (*Homo*), mouse (*Mus*), frog (*Xenopus*), and fruit fly (*Drosophila*) ERIS were obtained from the National Center for Biotechnology Information (NCBI). Dark gray indicates amino acid residues conserved in all these molecules; light gray indicates amino acid residues conserved among at least 3 species; and yellow indicates the putative ER retention sequences.

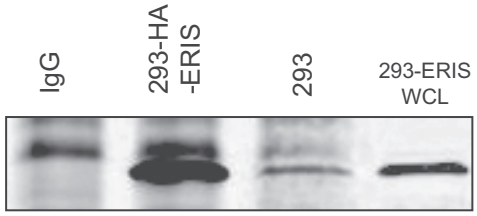

Fig. S3. 293 cells (293) or 293 cells stably expressing HA-ERIS (293-HA-ERIS) (1×10^6 cells/dish) were lysed, immunoprecipitated, and immunoblotted with anti-ERIS.

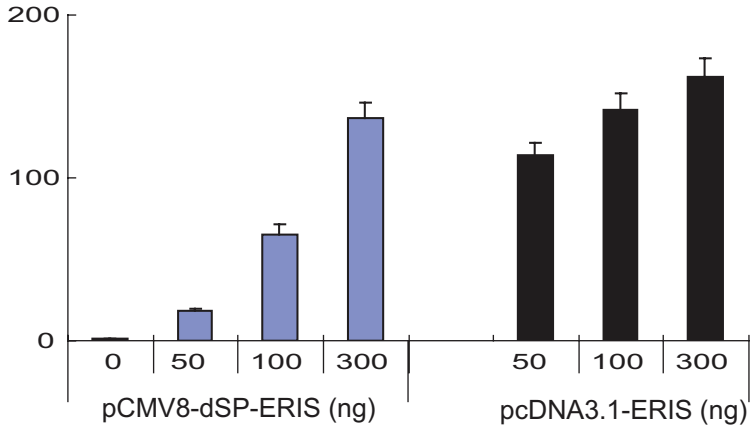

Fig. S4. 293 cells were transiently transfected with doses of 50 ng, 100 ng, and 300 ng p3 × Flag-CMV8-ERIS (*Left*) or pcDNA3.1-3′HA-ERIS (*Right*), along with 50 ng of IFN-β promoter. Fifty nanograms of TK was cotransfected as an internal control. Twenty-four hours after transfection, cells were analyzed for promoter activity by a reporter gene assay. Data represent mean ± SD. Similar results were obtained in 3 independent experiments.

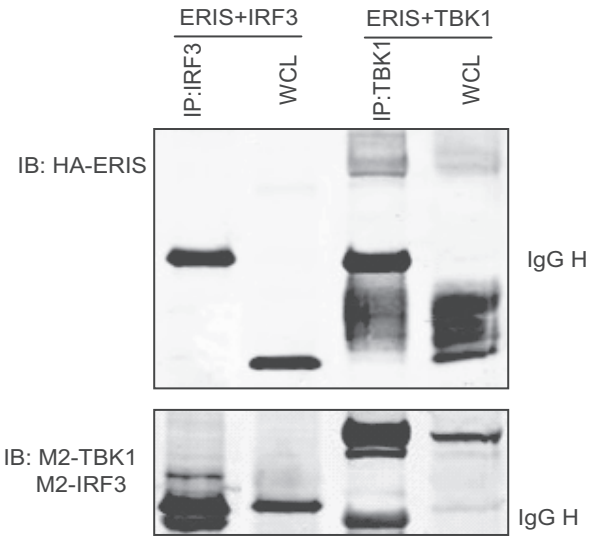

Fig. S5. 293 cells were transiently cotransfected with HA-ERIS and Flag-IRF3 (*Left*) or Flag-TBK1 (*Right*). Twenty-four hours later, cell lysates underwent immunoprecipitation (IP) with anti-Flag, followed by immunoblotting (IB) with anti-HA. Expression of Flag-IRF3 and Flag-TBK1 was verified by immunoblotting with anti-Flag.